1-19-00 A

PATENT

Attorney's Docket No.: U 012567-2

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Box Patent Application
Assistant Commissioner for Patents
Washington, D.C. 20231

NEW APPLICATION TRANSMITTAL

Transmitted herewith for filing is the patent application of Inventors:

1. SUMAN PREET **SINGH KHANUJA**
2. AJIT KUMAR **SHASANY**
3. SUNITA **DHAWAN**
4. MAHENDRA PANDURANG **DAROKAR**
5. SARITA **SATAPATHY**
6. TIRUPPADIRIPULIYUR R. **SANTHA KUMAR**
7. DHARMENDRA **SAIKIA**
8. NIRMAL KUMAR **PATRA**
9. JANAK RAJ **BAHL**
10. ARUN KUMAR **TRIPATHY**
11. SUSHIL **KUMAR**

WARNING: *The Declaration must name all of the actual inventor(s).*

For (title):
A NOVEL SCREENING METHOD FOR SELECTION OF INSECT TOLERANT PLANTS

1. Type of Application

This new application is for a(n) (check one applicable item below):

☑ Original (nonprovisional)

CERTIFICATION UNDER 37 CFR 1.10

I hereby certify that this New Application Transmittal and the documents referred to as enclosed therein are being deposited with the United States Postal Service on this date **JANUARY 18, 2000 in an envelope as "Express Mail Post Office to Addressee" Mailing Label Number EL386267046US addressed to the: Assistant Commissioner of Patents, Washington, D.C. 20231**

JENNIFER RASHKIN
(type or print name of person mailing paper)

Jennifer Rashkin
(Signature of person mailing paper)

NOTE: ***Each paper or fee referred to as enclosed herein has the number of the "Express Mail" mailing label placed thereon prior to mailing. 37 CFR 1.10(b).***

WARNING: ***Certificate of mailing (first class) or facsimile transmission procedures of 37 CFR 1.8 cannot be used to obtain a date of mailing or transmission for this correspondence.***

☐ Design

☐ Plant

WARNING: *Do not use this transmittal for a completion in the U.S. of an International Application under 35 U.S.C. 371(c)(4) unless the International Application is being filed as a divisional, continuation or continuation-in-part application.*

WARNING: *Do not use this transmittal for the filing of a provisional application.*

2. Benefit of Prior U.S. Application(s) (35 U.S.C. 119(e), 120, or 121)

NOTE: If the new application being transmitted is a divisional, continuation or a continuation-in-part of a parent case, or where the parent case is an International Application which designated the U.S., or benefit of a prior provisional application is claimed, then check the following item and complete and attach ADDED PAGES FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF PRIOR U.S. APPLICATION(S) CLAIMED.

WARNING: *If an application claims the benefit of the filing date of an earlier filed application under 35 U.S.C. 120, 121 or 365(c), the 20-year term of that application will be based upon the filing date of the earliest U.S. application that the application makes reference to under 35 U.S.C. 120, 121 or 365(c). (35 U.S.C. 154(a)(2) does not take into account, for the determination of the patent term, any application on which priority is claimed under 35 U.S.C. 119, 365(a) or 365(b).) For a c-i-p application, applicant should review whether any claim in the patent that will issue is supported by an earlier application and, if not, the applicant should consider canceling the reference to the earlier filed application. The term of a patent is not based on a claim-by-claim approach. See Notice of April 14, 1995, 60 Fed. Reg. 20,195, at 20,205.*

WARNING: *When the last day of pendency of a provisional application falls on a Saturday, Sunday, or Federal holiday within the District of Columbia, any nonprovisional application claiming benefit of the provisional **must** be filed prior to the Saturday, Sunday or Federal holiday within the District of Columbia. See 37 C.F.R. § 1.78(a)(3).*

☐ The new application being transmitted claims the benefit of prior U.S. application(s) and enclosed are ADDED PAGES FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF PRIOR U.S. APPLICATION(S) CLAIMED.

NOTE: If one of the following 3 items apply, then complete and attach ADDED PAGES FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF A PRIOR U.S. APPLICATION CLAIMED and a NOTIFICATION IN PARENT APPLICATION OF THE FILING OF THIS CONTINUATION APPLICATION.

☐ Divisional.

☐ Continuation.

☐ Continuation-in-Part (C-I-P).

3. Papers Enclosed That Are Required For Filing Date Under 37 CFR 1.53 (Regular) or 37 CFR 1.153 (Design) Application

20 Pages of specification (no page 10)

2 Pages of claims

1 Pages of Abstract

9 Sheets of drawing (3 SETS OF SHEETS 1-3)

☑ formal

☐ informal

WARNING: ***DO NOT** submit original drawings. A high quality copy of the drawings should be supplied when filing a patent application. The drawings that are submitted to the Office must be on strong, white, smooth, and non-shiny paper and meet the standards according to § 1.84. If corrections to the drawings are necessary, they should be made to the original drawing and a high-quality copy of the corrected original drawing then submitted to the Office. Only one copy is required or desired. Comments on proposed new 37 CFR 1.84. Notice of March 9, 1988 (1990 O.G. 57-62).*

NOTE: "Identifying indicia, if provided, should include the application number or the title of the invention, inventor's name, docket number (if any), and the name and telephone number of a person to call if the Office is unable to match the drawings to the proper application. This information should be placed on the back of each sheet of drawing a minimum distance of 1.5 cm. (⅝ inch) down from the top of the page." 37 C.F.R. 1.84(c).

(complete the following, if applicable)

☐ The enclosed drawing(s) are photograph(s), and there is also attached a "PETITION TO ACCEPT PHOTOGRAPH(S) AS DRAWING(S)". 37 C.F.R. 1.84(b).

4. Additional papers enclosed

☑ Preliminary Amendment

☐ Information Disclosure Statement (37 CFR 1.98)

☐ Form PTO-1449

☐ Citations

☐ Declaration of Biological Deposit

☐ Submission of "Sequence Listing," computer readable copy and/or amendment pertaining thereto for biotechnology invention containing nucleotide and/or amino acid sequence.

☐ Authorization of Attorney(s) to Accept and Follow Instructions from Representative

☐ Special Comments

☐ Other

5. Declaration or oath

☐ Enclosed

executed by *(check **all** applicable boxes)*

☐ inventors.

☐ legal representative of inventors. 37 CFR 1.42 or 1.43

☐ joint inventor or person showing a proprietary interest on behalf of inventor who refused to sign or cannot be reached.

☐ This is the petition required by 37 CFR 1.47 and the statement required by 37 CFR 1.47 is also attached. *See item 13 below for fee.*

☑ Not Enclosed.

WARNING: *Where the filing is a completion in the U.S. of an International Application but where a declaration is not available or where the completion of the U.S. application contains subject matter in addition to the International Application the application may be treated as a continuation or continuation-in-part, as the case may be, utilizing ADDED PAGE FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF PRIOR U.S. APPLICATION CLAIMED.*

☑ Application is made by a person authorized under 37 CFR 1.41(c) on behalf of *all the above named inventors.* (The declaration or oath, along with the surcharge required by 37 CFR 1.16(e) can be filed subsequently).

NOTE: It is important that all the correct inventor(s) are named for filing under 37 CFR 1.41(c) and 1.53(b).

☐ Showing that the filing is authorized. *(Not required unless called into question. 37 CFR 1.41(d).)*

6. Inventorship Statement

WARNING: *If the named inventors are each not the inventors of all the claims an explanation, including the ownership of the various claims at the time the last claimed invention was made, should be submitted.*

The inventorship for all the claims in this application are:

☐ The same

☐ Not the same. An explanation, including the ownership of the various claims at the time the last claimed invention was made,

7. Language

NOTE: An application including a signed oath or declaration may be filed in a language other than English. A verified English translation of the non-English language application and the processing fee of $130.00 required by 37 CFR 1.17(k) is required to be filed with the application or within such time as may be set by the Office. 37 CFR 1.52(d).

NOTE: A non-English oath or declaration in the form provided or approved by the PTO need not be translated. 37 CFR 1.69(b).

☑ English

☐ non-English

☐ the attached translation is a verified translation. 37 CFR 1.52(d).

8. Assignment

☑ An assignment of the invention to COUNCIL OF SCIENTIFIC & INDUSTRIAL RESEARCH

☐ is attached. A separate ☐ "COVER SHEET FOR ASSIGNMENT (DOCUMENT) ACCOMPANYING NEW PATENT APPLICATION" or ☐ FORM PTO 1595 is also attached.

☑ will follow.

NOTE: "If an assignment is submitted with a new application, send two separate letters—one for the application and one for the assignment." Notice of May 4, 1990 (1114 O.G. 77-78).

***WARNING:** A newly executed "CERTIFICATE UNDER 37 CFR 3.73(b)" must be filed when a continuation-in-part application is filed by an assignee. Notice of April 30, 1993. 1150 O.G. 62-64.*

9. Certified Copy

Certified copy of application

Country	Appln. No.	Filed

from which priority is claimed

☐ is attached.

☐ will follow.

NOTE: The foreign application forming the basis for the claim for priority must be referred to in the oath or declaration. 37 CFR 1.55(a) and 1.63.

NOTE: This item is for any foreign priority for which the application being filed directly relates. If any parent U.S. application or International Application from which this application claims benefit under 35 U.S.C. 120 is itself entitled to priority from a prior foreign application then complete item 18 on the ADDED PAGES FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF PRIOR U.S. APPLICATION(S) CLAIMED.

10. Fee Calculation (37 CFR 1.16)

A. ☑ Regular Application

Claims as Filed

Number Filed		Number Extra		Rate	Basic Fee 37 CFR 1.16(a) $690.00
Total Claims (37 CFR 1.16(c))	5 - 20 =	0	x $	18.00	
Independent Claims (37 CFR 1.16(b))	1 - 3 =	0	x $	78.00	
Multiple dependent claim(s), if any (37 CFR 1.16(d))			+ $	260.00	

☐ Amendment cancelling extra claims enclosed.

☐ Amendment deleting multiple-dependencies enclosed.

☐ Fee for extra claims is not being paid at this time.

NOTE: If the fees for extra claims are not paid on filing they must be paid or the claims cancelled by amendment, prior to the expiration of the time period set for response by the Patent and Trademark Office in any notice of fee deficiency. 37 CFR 1.16(d).

Filing Fee Calculation $

B. ☐ Design application ($310.00 — 37 CFR 1.16(f))

Filing Fee Calculation $

C. ☐ Plant application ($480.00 — 37 CFR 1.16(g))

Filing Fee Calculation $

11. Small Entity Statement(s)

☐ Verified Statement(s) that this is a filing by a small entity under 37 CFR 1.9 and 1.27 is(are) attached or has been filed.

Filing Fee Calculation (50% of **A**, **B** or **C** above) $

NOTE: Any excess of the full fee paid will be refunded if a verified statement and a refund request are filed within 2 months of the date of timely payment of a full fee. 37 CFR 1.28(a).

12. Request for International-Type Search (37 CFR 1.104(d)) *(Complete, if applicable)*

☐ Please prepare an international-type search report for this application at the time when national examination on the merits takes place.

13. Fee Payment Being Made At This Time

☑ Not Enclosed

☑ No filing fee is to be paid at this time. *(This and the surcharge required by 37 CFR 1.16(e) can be paid subsequently.)*

☐ Enclosed

☐ basic filing fee $

☐ Recording assignment
($40.00; 37 CFR 1.21(h)) (See attached "COVER SHEET FOR ASSIGNMENT ACCOMPANYING NEW APPLICATION.")

☐ Petition fee for filing by other than all the inventors or person on behalf of the inventor where inventor refused to sign or cannot be reached.
($130.00; 37 CFR 1.47 and 1.17(h)) $

☐ For processing an application with a specification in a non-English language.
($130.00; 37 CFR 1.52(d) and 1.17(k)) $

☐ Processing and retention fee
($130.00; 37 CFR 1.53(d) and 1.21(l))

☐ Fee for international-type search report
($40.00; 37 CFR 1.21(e)). $

NOTE: 37 CFR 1.21(l) establishes a fee for processing and retaining any application which is abandoned for failing to complete the application pursuant to 37 CFR 1.53(d) and this, as well as the changes to 37 CFR 1.53 and 1.78, indicate that in order to obtain the benefit of a prior U.S. application, either the basic filing fee must be paid or the processing and retention fee of §1.21(l) must be paid within 1 year from notification under §53(d).

Total fees enclosed $

14. Method of Payment of Fees

☐ Check in the amount of $

☐ Charge Account No. 12-0425 in the amount of $

A duplicate of this transmittal is attached.

NOTE: Fees should be itemized in such a manner that it is clear for which purpose the fees are paid. 37 CFR 1.22(b).

15. Authorization to Charge Additional Fees

WARNING: *If no fees are to be paid on filing, the following items should <u>not</u> be completed.*

WARNING: *Accurately count claims, especially multiple dependent claims, to avoid unexpected high charges, if extra claim charges are authorized.*

☐ The Commissioner is hereby authorized to charge the following additional fees by this paper and during the entire pendency of this application to Account No. 12-0425.

☐ 37 CFR 1.16(a), (f) or (g) (filing fees)

☐ 37 CFR 1.16(b), (c) and (d) (presentation of extra claims)

NOTE: Because additional fees for excess or multiple dependent claims not paid on filing or on later presentation must only be paid or these claims cancelled by amendment prior to the expiration of the time period set for response by the PTO in any notice of fee deficiency (37 CFR 1.16(d)), it might be best not to authorize the PTO to charge additional claim fees, except possibly when dealing with amendments after final action.

☐ 37 CFR 1.16(e) (surcharge for filing the basic filing fee and/or declaration on a date later than the filing date of the application)

☐ 37 CFR 1.17 (application processing fees)

WARNING: *While 37 CFR 1.17(a), (b), (c) and (d) deal with extensions of time under §1.136(a), this authorization should be made only with the knowledge that: "Submission of the appropriate extension fee under 37 C.F.R. 1.136(a) is to no avail <u>unless</u> a request or petition for extension is filed." (Emphasis added). Notice of November 5,1985 (1060 O.G. 27)*

☐ 37 CFR 1.18 (issue fee at or before mailing of Notice of Allowance, pursuant to 37 CFR 1.311(b))

NOTE: Where an authorization to charge the issue fee to a deposit account has been filed before the mailing of a Notice of Allowance, the issue fee will be automatically charged to the deposit account at the time of mailing the notice of allowance. 37 CFR 1.311(b).

NOTE: 37 CFR 1.28(b) requires "Notification of any change in loss of entitlement to small entity status must be filed in the application ... prior to paying, or at the time of paying, ... issue fee". From the wording of 37 CFR 1.28(b): (a) notification of change of status must be made even if the fee is paid as "other than a small entity" and (b) no notification is required if the change is to another small entity.

16. Instructions As To Overpayment

☐ credit Account No. 12-0425

☐ refund

Signature of Attorney

Reg. No. 33,778

Tel. No. (212) 708-1935

Janet I. Cord
Ladas & Parry
26 West 61 Street
New York, NY 10023

☐ **Incorporation by reference of added pages**

(Check the following item if the application in this transmittal claims the benefit of prior U.S. application(s) (including an international application entering the U.S. stage as a continuation, divisional or C-I-P application) and complete and attach the ADDED PAGES FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF PRIOR U.S. APPLICATION(S) CLAIMED)

☐ Plus Added Pages for New Application Transmittal Where Benefit of Prior U.S. Application(s) Claimed

Number of pages added ___

☐ Plus Added Pages for Papers Referred to in Item 4 Above

Number of pages added ___

☐ Plus "Assignment Cover Letter Accompanying New Application"

Number of pages added ___

☑ **Statement Where No Further Pages Added**

(If no further pages form a part of this Transmittal, then end this Transmittal with this page and check the following item:)

☑ This transmittal ends with this page.

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application: SUMAN PREET **SINGH KHANUJA**, et al

For: A NOVEL SCREENING METHOD FOR SELECTION OF INSECT TOLERANT PLANTS

Attorney Docket No.: U 012567-2

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

PRELIMINARY AMENDMENT

Please amend the above application as follows:

IN THE CLAIMS

Claim 4, line 1, delete "or 3"

Claim 5, line 1, delete "or 3"

Respectfully submitted,

JANET I. CORD
LADAS & PARRY
26 WEST 61ST STREET
NEW YORK, NEW YORK 10023
REG.NO.33778(212)708-1935

CERTIFICATE UNDER 37 1.10

I hereby certify that this paper is being deposited with the United States Postal Service on this date JANUARY 18, 2000 in an envelope as "EXPRESS MAIL POST OFFICE TO ADDRESSEE" Mailing Label Number EL386267046US addressed to the: Commissioner of Patents and Trademarks, Washington, D.C. 20231

JENNIER RASHKIN
(Type or print name of person mailing paper)

(Signature of person mailing paper)

NOTE: Each paper or fee referred to as enclosed herein has the number of the "EXPRESS MAIL" mailing label place thereon prior to mailing 37 CFR 1.16(b).

Field of invention:

The present invention relates to a novel method for screening, identifying and developing insect tolerant plant, genotypes or clones. The invention is the result of development of a novel devised procedure for early selection of somaclonal variants among the *in vitro* raised large population of a menthol rich superior variety of *Mentha arvensis* leading to generation of an insect tolerant mint plant with high oil and menthol yield. The plant can be propagated vegetatively by suckers and is genetically stable for commercial cultivation. The plant type is unique with the extra wide canopy and height surpassing all existing varieties permitting better sunlight capture and vigorous foliage production ultimately giving high oil and menthol yield. The procedure although applied to *Mentha* against lepidopteran insect pests can be employed for any plant of choice against all damaging insect pests.

Background of the invention:

Mentha arvensis Linn. var *piperescens*. Holmes (menthol or Japanese mint) is an industrial crop that is widely cultivated for its essential oil from which menthol is purified by crystallization through freezing. Menthol and other terpenoids present in the dementholated oil of *Mentha arvensis* are variously used in the food, perfumery and pharmaceutical industries. In the varietal improvement programme the genetic alternations leading to enhancement in the tolerance against pest and disease and improving other adaptive characters determining the yield and quality of essential oil is most desirable. Insect tolerance is desired particularly against *Spilarctia obliqua* in mentha, which is the most damaging pest of mints in India. Since the mints are of considerable interest to the industrial world, selection programs for the isolation of desirable clones with improved terpene accumulation and suitable agronomic traits are being pursued in several laboratories. However, due to the inherent problem of seed setting in mints, conventional breeding programmes are severely hampered. *In vitro* high efficiency procedures for cell and callus cultures and shoot regeneration from axillary buds and leaf explants have been reported in some species of the genus *Mentha*, especially the commercially important species *M. piperata* and *M. spicata*.

However, in *M. arvensis* mostly the media used have permitted shoot proliferation from limited explants such as nodes, terminal and axillary buds and distal segments of leaf petiole at relatively low levels of efficiency. Attempts to develop new varieties or genotypes by alternative methods like clonal selection, mutation breeding and somaclonal variation have been followed in mints (S. P. S. Khanuja, A. K. Shasany, S. Dhawan, S. Kumar, Rapid procedure for isolating somaclones of altered genotypes in *Mentha arvensis*. J Med. Aroma. Plant Sci. 20 (1998) 359-361.). The applicants have reported high efficiency protocols for rapid detection and selection procedure for development of somaclonal variants through molecular approaches in *Mentha arvensis* (S. P. S. Khanuja, A. K. Shasany, S. Dhawan, S. Kumar, Rapid procedure for isolating somaclones of altered genotypes in *Mentha arvensis*. J Med. Aroma. Plant Sci. 20 (1998) 359-361). We have also successfully defined the conditions and media to restrict the emergence of somaclonal variation for stable micropropagation purposes (A. K. Shasany, S. P. S. Khanuja, S. Dhawan, U. Yadav, S. Sharma, S. Kumar, High regenerative nature of *Mentha arvensis* internodes. Journal of Biosciences 23 (1998) 641-646.).

In the present invention, the applicants have utilized the already reported protocol (S. P. S. Khanuja, A. K. Shasany, S. Dhawan, S. Kumar, Rapid procedure for isolating somaclones of altered genotypes in *Mentha arvensis*. J Med. Aroma. Plant Sci. 20 (1998) 359-361.) to generate and capture the somaclonal variations in larger frequencies. We, then screened the somaclones for their tolerance to *Spilarctia obliqua* by devising a novel method for rapid and dependable selection of tolerant clone(s) right at the tissue culture stage. Further the tolerant plants, which survived were tested for their stability, essential oil, menthol content and biomass yield. Consequently, the plant GRB2-18 was selected for unmatchable vigor of shoots, higher menthol production and increased insect tolerance through field evaluation.

Brief Description of accompanying drawings :

1. Photographs 1 and 2 of drawing sheet 1 show field view of morphological features of the plants of the present invention at 70 days and 100 days respectively.
2. Photographs 1 and 2 of drawing sheet No. 2 show the rapid growth of the 'Sambhav' plant and its canopy at 70 days and 100 days respectively.
3. Photograph 1 of drawing sheet No.3 shows instar larvae infected individual clones of other plants in comparison with clone of 'Sambhav'.
4. Photograph 2 of drawing sheet No.3 shows the unique RAPD profile of 'Sambhav'.

Detailed description:

Breeding history :

Bihar hairy caterpillar (*Spilarctia obliqua*) is a major pest of polyphagus nature which infects heavily over a large area of mint crops frequently in Terai and north Indian plains. The infestation sometimes is so heavy and unmanageable that it may lead to more than 80% to complete loss of foliage and consequently the oil yield proportionately. Hence it is desirable to explore the possibility of developing insect tolerant high yielding clones in mints. The popular menthol mint variety "Himalaya" developed in 1996 by CIMAP (US Pat. No PP10935) was, therefore, used as the starting material for specific improvement towards insect tolerance through generation of somaclonal variation and simultaneously looking for improved plant type with higher essential oil and menthol yields.

Encouraged by the initial leads in the laboratory for rapid detection and isolation of somaclonal variants by using the protocol reported by us (S. P. S. Khanuja, A. K. Shasany, S. Dhawan, S. Kumar, Rapid procedure for isolating somaclones of altered genotypes in *Mentha arvensis*. J Med. Aroma. Plant Sci. 20 (1998) 359-361), we generated 3000 independent somaclones. These clones were subjected to detection of molecular variation at the tissue culture stage itself through RAPD profiling. DNA

was isolated from 40 mg of leaf tissue and Polymerase chain reactions (PCRs) were carried out in 25 µl volume. A reaction tube contained 25 ng of DNA, 0.2 unit of Taq DNA polymerase , 100 µl each of dNTPs , 1.5 mM $MgCl_2$ and 5 p mol of decanucleotide primers. The amplifications were carried out using a thermal cycler (MJ Research, USA). The amplified products were loaded in 1.2% agarose gel containing 0.5 µg ml^{-1} of ethidium bromide and photographed by Polaroid system. Twelve decamer primers having the sequences AAATCGGAGC, GTCCTACTCG, GTCCTTAGCG, TGCGCGATCG, AACGTACGCG, GCACGCCGGA, CACCCTGCGC, CTATCGCCGC, CGGGATCCGC, GCGAATTCCG, CCCTGCAGGC, CCAAGCTTGC were used to analyse all the *in vitro* regenerated clones. Out of 3000 regenerated clones 245 showed variation at DNA level in the RAPD profiles compared to the control plant "Himalaya".

The individual molecular variants selected through RAPD analysis of somaclones as above were then subjected to screening against the larvae of lepidopteran insect pest *Spilarctia obliqua* .

For this purpose, a new strategy was devised by subjecting the *in vitro* growing clones to attack by actively feeding 3rd instar larvae by releasing them right in the culture tubes containing individual clones on the rooting medium (Sheet # 3, Photograph # 1). Most of the shoots of the clones were eaten away by these larvae within 2-3 days. However, three clones showed the least feeding by the larvae. In these tubes, only initial bites could be observed and nonfeeding was also conspicuous by typical symptoms of stalled growth in the starved larvae. These larvae were then transferred to other clone tubes, where they resumed feeding. This led to the applicants to believe that the three clones must have some characteristics not liked by the feeding larvae. So the applicants again confirmed this by releasing another set of actively feeding 4th instar larvae into the tubes containing these three identified clones. This process was repeated three times and each time, the larvae showed non-preference and stopped feeding.

The applicants then hardened these three "insect-non-preferred" clones namely, CIMAP/GRB 1-06, 2-18 and 5-15 and transferred to the glasshouse in pots. Among these three clones, CIMAP/GRB 2-18 showed conspicuously vigorous growth

characteristics attaining much higher height and shoot proliferation. This clone GRB 2-18 was multiplied *in vitro* from internodal explants through stable micropropagation protocol developed in the laboratory (A. K. Shasany, S. P. S. Khanuja, S. Dhawan, U. Yadav, S. Sharma, S. Kumar, High regenerative nature of *Mentha arvensis* internodes. Journal of Biosciences. 23 (1998) 641-646.) for genetic uniformity into about 1000 plantlets. Randomly 100 regenerated shoots from the clone were tested for variation in their profiles using the above described 12 random primers. Complete uniformity was observed among these clones without any variation from the control mother plant GRB 2-18 but the profiles were clearly distinct from other mint varieties including "Himalaya".

The overall objective of the invention was not only to develop insect tolerant genotypes but simultaneously also have better plant type with high menthol content and herbage yield for better productivity. So, the plant of invention was tested in field trial for oil yield, menthol content and herbage production against the checks (varieties developed by CIMAP). Replicated field trials were conducted following normal agronomic practices by planting multiplied suckers in the month of January, 1998 and 1999 for 2 consecutive years in RBD fashion and different growth and yield characteristics were recorded (Table 1). For field trials 10m X 10m plots were prepared by adding only FYM 1.5 ton per ha. Astonishingly the plant CIMAP/GRB 2-18 was able to out-compete all existing varieties in its rate of growth. It was so rapidly growing that it could cover the inter-row spacing of 80cm completely within a period of 90-100 days, which was not the case of other control varieties taken (Sheet # 2, Photograph # 1 and 2). The plant canopy covered a space of 85 to 90 cm in 110 days in comparison to Himalaya(62-70 cm), Shivalik (50-56 cm), Gomti (70-75), Kosi (65-72), Kalka (40-60 cm) and MAS-1(40-50 cm).This was the most desirable advantage to the plant as it produced highest amount of herbage and ultimately the oil and menthol.

Natural infestation of *Spilarctia obliqua* in field in absence of insecticidal spray was scored in both 1998 and 1999 crop stands. The clone GRB 2-18 had less than 10% leaf damage compared to 86% in case of Gomti, 58% in Himalaya, 50% in Kosi in the year 1998. Similar trend was also recorded in 1999 when leaf damage was 70%, 50%

and 46% for Gomti, Himalaya and Kosi, respectively compared to 6% in case of GRB 2-18. This plant of invention also produced highest amount of essential oil per unit area in comparison to the other control plant in both the successive yield trials. The total menthol yield consequently, was the highest due to more oil and herbage yield (Table 1). The plant was hence named "Sambhav" (means "Possible") that's so because the expression of its genotype made this rare but most desirable combination of traits of high level insect tolerance and plant type with rapid growth possible in a single plant.

Thus the plant of invention "Sambhav" combines expression of the character of very high tolerance to *Spilarctia obliqua* attack, high growth rate and regenerability while producing highest total herbage, oil and menthol yield per unit area in comparison to the other control plants and thereby being unique, novel genotype which can be exploited commercial cultivar superior to other available mint varieties for menthol production.

Accordingly, the invention provides a novel procedure to screen, identify and develop insect tolerant plant genotypes or clones wherein,

a. the clones are generated through tissue culture as somaclones and their molecular distinctiveness is established prior to screening through RAPD analysis at *in vitro* stage itself,
b. the clones could be generated vegetatively in tissue culture, glasshouse or field by asexual reproduction methods,
c. the identified molecular variants are micropropagated for multiplication and checked for the stability at molecular level through RAPD among clones of larger population,
d. the identified stable variants after being multiplied are transferred to individual culture tubes for forced feeding of insects by releasing actively feeding larvae or nymphs into each culture tube,
e. the surviving clones are *in-vitro* multiplied and rechecked for insect larval non-preference and then field evaluated under natural or artificial insect infestation conditions,

f. the procedure can be followed for mints and all other plants where raising somaclones or rapid propagation is amenable, and

g. the larvae are lepidopteran but scope of invention is not limited to larvae of lepidopteran and can be any actively feeding stage of pests.

In an embodiment, the plants are raised by raising somaclones or rapid propagation method.

In another embodiment, a novel screening method useful for the selection and development of an insect tolerant mint plant genotypes or clones, said method comprising the steps of:

a) generating the clones of an insect tolerant plant through tissue culture as somaclones and establishing their molecular distinctiveness prior to screening through RAPD analysis at in *vitro* stage itself;

b) micro-propagating the identified molecular variants for multiplication and checking for the stability at molecular level through RAPD among clones of larger population,

c) transferring the identified stable variants after being multiplied to individual culture tubes for forced feeding of insects by releasing actively feeding larvae or nymphs into each culture tube,

d) multiplying in-vitro the surviving clones and rechecking for insect larval non-preference and then field evaluating under natural or artificial insect infestation conditions.

In still another embodiment, the scope of invention is not limited to larvae of lepidopteran and used and can be any actively feeding stage of pests.

In a further embodiment, the clones could be generated vegetatively in tissue culture, glass house or field by asexual reproduction method.

The invention in another embodiment provides a new and distinct mint plant of *Mentha arvensis* 'Sambhav' which is covered in a co-pending U.S. Patent Application

Sl.No. ....., developed through tissue culture, possessing the following combination of characters:

a. the plant is highly tolerant to the insect pest *Spilarctia obliqua* ,
b. the tolerance trait of the plant is not limited to *Spilarctia obliqua* which could be against other actively foliage feeding insect pests also,
c. the plant possesses vigorous and rapid vegetative growth with high regenerability covering at least 85 cm canopy area and a height of at least 73.5cm in a maximum of 100 days,
d. the plant has distinct molecular profile by random amplified polymorphic DNA (RAPD) using 20 OPJ primers and 20 MAP primers distinguishing the plant from the other existing varieties (Drawing sheet # 3, Photograph # 2),
e. the plant shows tolerance to leaf spot, rust and powdery mildew as in the parent variety 'Himalaya',
f. the plant has characteristic light greenish leaves, whitish flowers with distinct morphology of single main stem with branches coming out of the lower nodes imparting a shape of up-side down open filled umbrella to the canopy allowing equal distribution of sunlight, thus prevents yellowing and fall of lower leaves (Drawing sheet # 1, Photograph # 1 and 2),
g. the plant is able to produce highest herbage, oil and menthol yield per unit area as compared to all other existing varieties.

Table 1. Comparative growth and yield characteristics of plant of invention "Sambhav" (GRB 2-18) in relation to the existing Japanese mint varieties

Property	Himalaya	Shivalik	MAS-1	Kalka	Gomti	Kosi	Sambhav
Plant height (cm)	60.9+6.2	68.25+3.4	43.2+1.1	51.6+2.7	74.2+7.3	66.2+3.1	73.5+3.3
Canopy (cm)	62-70	50-56	40-50	40-60	70-75	65-72	85-90
Leaf number	40.2+4.2	49.6+4.8	40.0+1.4	38+2.0	46.0+2.8	50.4+3.8	44.2+2.2
Branch length	40.4+6.8	44.2+8.7	36.6+9.0	45+9.7	58.4+14.7	52.0+5.7	50.1+8.8
Branch number	29.2+4.0	22.4+1.7	13.6+1.7	27.2+1.1	28.4+2.9	30.4+1.7	35.1+2.8
Leaf length	7.1+0.5	7.3+0.3	7.5+1.0	7.4+0.8	7.7+0.6	7.40+0.7	8.6+0.6
Leaf breadth	4.5+0.2	5.0+0.2	3.8+0.5	4.0+0.3	4.7+0.2	4.60+0.5	5.50+0.3
Petiole length	1.75+0.3	1.40+0.1	1.6+0.5	1.5+0.5	1.4+0.1	1.4+0.3	1.70+0.2
Oil %	0.7	0.5	0.5	0.8	0.5	0.8	0.8
Menthol%	80	77	88	82	73	76	77
Herbage yield (Q per 100 m^2)	1.25	0.75	0.50	0.80	1.60	1.70	2.10
Oil yield (Kg per 100 m^2)	0.85	0.35	0.27	0.64	0.83	1.42	1.61

Taxonomic description of the mint plant "Sambhav"

1. Genus : ***Mentha***
2. Species : ***arvensis*** L.
3. Family : Lamiaceae
4. Common name : Japanese mint/corn mint/ menthol mint
5. Plant height : 73.5±3.27 cm.
6. Plant canopy : 85-90 cm
7. Growth habit : Erect sturdy main stem, profuse synchronous branching with growth habit giving an up-side-down open filled umbrella shape to the canopy (Drawing sheet # 1, Photograph # 1 and 2).
8. Stem : Round to quadrangular hard, woody, green (144A), faint purplish red (59B) pigmentation at the base, 5-10 mm thick at 5th internode.
9. Leaf :

Colour	Light green(137B)
Texture	Moderately thick and rigid
Surface	Hairy and rough
Shape	Lacerate
Margin	Moderately deep serration (14 to 50 number)
Tip	Acute
Base	Attenuate
Size	Moderately broad
Petiole length	1.7 ± 0.17 cm
Area	16.1 cm^2 (Average of full branch)
Length	8.56 ± 0.56 cm
Width	5.51 ± 0.34 cm

10. Leaf : stem ratio (w/w) : 1.7

11. Inflorescence : Indefinite recemose

Total number of florets 20 to 35

12. Flowers

Arranged in whorls surrounding the stem at the base of lateral leaves.

Pedicel	Yellow green (145C)
Calyx	Four, Yellow green (145B)
Corolla	Purplish white (76D), four, fused to a bell shaped corolla tube
Anthers	Four, ocidimetary, come out of the corolla tube
Stigma	Bifid, Purple (76 A)

13. Oil content in the fresh herb (%) : 0.77 to 0.8

14. Oil quality :

Menthol content (%) : 75 to 80 %

Congealing point : 2 to 21 °C

15. Herbage (Shoot biomass Q/ 100 m^2) : 2.10

Table 2. Comparison of "Sambhav" with other existing varieties of the same botanical and market class of *Mentha arvensis*

Character	cv.MAS-1	cv.Kalka	cv.Shivalik	cv.Gomti	cv.Himalaya	cv Sambhav
1. Leaf : stem ratio	1.2	1.0	0.8	1.0	1.5	1.7
2. Stem						
•colour	Upper green (141C) , lower pigmented red purple(71B)	GREEN(141C), lower pigmented red purple(71B)	Green(141B) lower pigmented red purple(71B)	Green(143B) lower pigmented purple(77A)	Green(143C) lower purplish(70A)	Green(144A) lower purplish(59B)
•Stiffness	Hard	Hard	Woody	Woody	Hard	Woody
•Thickness at 5th internode(mm)	6.8	3.6	10.0	7.9	8.0	10.0
3. Leaf						
•Colour	Green(139C)	Green(139C)	Green(138A)	Green(138A)	Green(138B)	Green(137B)
•Length(cm)	6.46	6.7	5.2	6.4	6.2	8.6
•Width(cm)	2.3	2.4	2.8	3.1	3.6	5.1
•Area (cm^2)	8.2	8.7	10.1	13.3	15.4	16.1
4. Petiole length (cm)	1.1	1.2	0.6	0.7	1.5	1.7
5. Flower colour	Whitish	Whitish	Whitish	Whitish	Pinkish white	Purplish white (76C)
6. Flower length (mm)	3.72	3.48	4.66	4.74	3.72	4.65
7. Calyx colour	Green (143B) with red purple streaks (71B)	Green (143B) with red purple streaks (71B)	Green (141B)	Green (141B)	Green (143C)	Green (145B)
8. Stigma colour	White	White	Purplish (71C)	Purplish (71C)	White	Purple (76A)
9. Disease incidence to						

• Rust Tolerant	Resistant	Susceptible	Susceptible	Resistant	Resistant	
• Alternaria leaf blight	-	Resistant	Susceptible	Susceptible	Resistant	Resistant
• Corynespora leaf spot	Susceptible	Tolerant	Susceptible	Susceptible	Tolerant	Tolerant
• Powdery mildew	Susceptible	Susceptible	Tolerant	Tolerant	Tolerant	Tolerant
10. Pest infestation						
Spilarctia obliqua	Susceptible	Susceptible	Susceptible	Highly Susceptible	Susceptible	Tolerant

The plant genotype "Sambhav" developed in the present invention is a herbaceous perennial with a single tall upright stem possessing several lateral branches coming out from the lower nodes laterally rising in a fashion to give a shape of an open filled umbrella turned upside down.. This special arrangement of branches facilitates the distribution of the captured sunlight equally to all the leaves and hence avoiding shading thereby, reducing lower leaf fall amounting to the prevention of economic loss to the plant. The chromosome number of the plant is 2n=96. The colour codes are in accordance with the "RHS colour chart published by the Royal Horticultural Society, 80 Vincent Square, London SW1P 2PE,1995.

Evidence of uniformity and stability

No variants of any kind (morphological or molecular) has been observed since 1997 indicating the stability and uniformity of the genotype. Further, the comparative herbage and oil yields of "Sambhav" were significantly higher in comparison to other varieties/genotypes in different years and seasons. Due to vigorous vegetative growth this genotype can be harvested earlier without reducing the yield of herbage, oil or menthol. The traits of insect tolerance against *S. obliqua* is unprecedented and stable.

Statement of distinction

The genotype "Sambhav" possessing a very high level of insect tolerant character against leaf damage by *S. obliqua* larvae is unique and unprecedented not possessed by any known variety. Additionally, it has a distinct canopy of one straight main stem with many lower branches arranged like an open filled umbrella turned upside down which is characteristic to this genotype only. The genotype is having highest biomass and highest oil yield unit area in comparison to others. The total menthol yield of the new genotype is higher per unit area in comparison to other genotypes. Its genetic make up is distinct in terms of DNA profile.

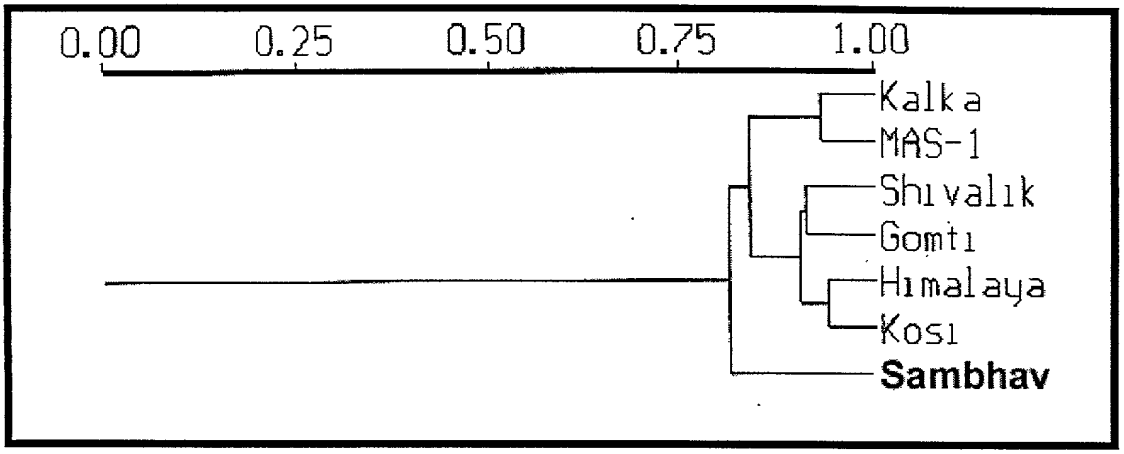
Randomly Amplified Polymorphic DNA analysis : The RAPD profiles of the plant "Sambhav" were unambiguously able to establish its distinct identity as completely different from the parent plant "Himalaya" as well as the known released

varieties. The plant of the present invention was developed by screening molecular variants among somaclones already differentiated as distinct, unique and novel at DNA level. The plant is having desirable morphological and economical traits in a rare unmatchable combination and is available only with us in CIMAP. No variation in the RAPD patterns was observed in the analysis of the micropropagated as well as field raised population in successive generations indicating the stability of the genotype. The 20 MAP primers (MAP 01 to MAP 20) with the sequence AAATCGGAGC, GTCCTACTCG, GTCCTTAGCG, TGCGCGATCG, AACGTACGCG, GCACGCCGGA, CACCCTGCGC, CTATCGCCGC, CGGGATCCGC, GCGAATTCCG, CCCTGCAGGC, CCAAGCTTGC, GTGCAATGAG, AGGATACGTG, AAGATAGCGG, GGATCTGAAC, TTGTCTCAGG, CATCCCGAAC, GGACTCCACG, AGCCTGACGC and 20 OPJ primers (Operon Technologies Inc, USA) were used for the analysis and similarity indices were computed to generate similarity matrix among existing varieties and the plant Sambhav (Table 3). The OPJ primers (01 to 20) were procured from Operon technologies, USA. The MAP primers were used to develop a unique and distinct RAPD profile (Drawing sheet # 3, Photograph # 2)of the Plant.

Table 3. Similarity indices of different control plants analysed in comparison to Sambhav

Gomti	Himalaya	Kosi	MAS-1	Kalka	Shivalik	Sambhav
1.00						
0.90	1.00					
0.89	0.94	1.00				
0.91	0.92	0.92	1.00			
0.85	0.88	0.85	0.89	1.00		
0.92	0.93	0.91	0.90	0.87	1.00	
0.87	0.73	0.78	0.82	0.83	0.84	1.00

Figure 1: Genetic similarity of the new clone GRB 2-18 with other varieties of *Mentha arvensis*.



From RAPD analysis the profiles were studied and similarity indices were calculated which were put into a matrix. This matrix was used to produce a graphic phenogram by means of UPGMA (unweighted pair group method with arithmetic average) cluster analysis (Figure 1). As represented in the phenogram the clone of the invention is quite different from the other varieties. The diversity of the genotype of the plant of invention 'Sambhav' is 13%, 28%, 22%, 18%, 17% and 16% from the varieties Gomti, Himalaya, Kosi, MAS-1, Kalka and Shivalik. Highest 28% difference in terms of polymorphic profiles was observed for the genotype of the invention with the parent plant Himalaya from which it was developed as a somaclone. The plant genotype of the invention 'Sambhav' was most similar in terms of polymorphism with Gomti. In total the distinctiveness of the clone in RAPD profiles was established as total polymorphism detected .

So the protocol for developing insect tolerant plants *in vitro* can be explained in details as an example of the development of 'Sambhav' which involves following steps and sub-steps.

Example of development of insect tolerant plant 'Sambhav'.

Step A: Explant, culture conditions and regeneration for generating genetically modified somaclones.

1. The *Mentha arvensis* cv Himalaya explant material was collected from the field grown plants and washed sequentially with 2% detergent, distilled water containing a few drops of Savlon (Johnson and Johnson, India), 0.1% acidified mercuric chloride and autoclaved distilled water. About 1cm long internode pieces were inoculated in the MS media (Murashige T, Skoog F. 1962. A revised medium for rapid growth and bioassay with tobacco tissue cultures. *Physiol. Planta.* **15**:473-497.) containing the auxin, 0.2μg ml^{-1} 2,4 dichlorophenoxy acetic acid (2, 4-D) and the cytokinin, 7μg ml^{-1} 6-(γγ'- dimethylallyl amino) purine (2iP or 2aP).
2. The cultures were grown at 25±2°C and 400 to 600 lux light intensity with 16 h photoperiod.
3. The regenerated shoots were separated at 12 weeks from the explant inoculation and transferred to the MS medium free of hormones for rooting.
4. The plantlets thus generated were examined for any genotypic change by comparing their RAPD profiles with that of cv Himalaya using the 12 random decanucleotide primers having the sequences AAATCGGAGC, GTCCTACTCG, GTCCTTAGCG, TGCGCGATCG, AACGTACGCG, GCACGCCGGA, CACCCTGCGC, CTATCGCCGC, CGGGATCCGC, GCGAATTCCG, CCCTGCAGGC, CCAAGCTTGC.
5. We obtained 245 molecular variants after screening 3000 regenerated clones. Out of 245 clones 155 clones were different from 'Himalaya' with one primer out of the 12 primer used. Eighty-two variants were different from 'Himalaya' with two primers and the rest 8 variants with three or more primers.

Step B: Screening genetically modified somaclones for *Spilarctia obliqua* tolerance

1. The individual molecular variants selected through RAPD analysis of regenerated clones were multiplied from the internode segments, which were inoculated in MS based media containing vitamins,100 μg ml^{-1} myo-inositol, 3% wv^{-1} sucrose, 1.5% wv^{-1} agar (Difco), 0.5 μg ml^{-1} NAA (1-napthalene acetic acid) and 5 μg ml^{-1} BAP (6-benzyl aminopurine).
2. The cultures were grown at 25±2°C and 400 to 600 lux light intensity with 16 h photoperiod.
3. These clones from individual variants were tested for uniformity through RAPD profiling after isolating DNA from 40mg of tissue, using the random primers (AAATCGGAGC, GTCCTACTCG, GTCCTTAGCG, TGCGCGATCG, AACGTACGCG, GCACGCCGGA, CACCCTGCGC, CTATCGCCGC, CGGGATCCGC, GCGAATTCCG, CCCTGCAGGC, CCAAGCTTGC). The clones were found to be similar in their profiles in comparison to the parental genetically distinct somaclones but were distinctly different from the Plant 'Himalaya'.
4. These regenerated shoots were grown in MS medium without growth hormones for rooting for 12 weeks.
5. The variants were then subjected to screening against the larvae of lepidopteran insect pest *Spilarctia obliqua* in replicates.
6. The *in vitro* growing genetically distinct clones were subjected to feeding attack by actively feeding 3rd instar larvae by releasing them right in the culture tubes containing individual clones on the rooting medium (Drawing sheet # 3, Photograph # 1). Most of the shoots of the clones were eaten away by these larvae within 2-3 days. However, three clones clones (CIMAP/GRB 1-06, 2-18 and 5-15) showed the least feeding by the larvae. In these tubes, only initial bites could be observed and non-feeding was also conspicuous by typical symptoms of no growth in the starved larvae.

7. These larvae were then transferred to other clone tubes, where they resumed feeding. This led to us to believe that the three clones must have some characteristics not liked by the feeding larvae.
8. We again confirmed this by releasing another set of actively feeding 4th instar larvae into the tubes containing these three identified clones.
9. This was repeated three times and each time, the larvae showed non-preference and stopped feeding.
10. These three clones were then hardened and planted in the earthen pots in the glasshouse and observed initially for the growth characters. The growth of the clone GRB 2-18 was more vigorous compared to the other clones and the parent plant Himalaya.
11. These were covered with nets individually and 10 insect larvae per plant were released to assay the leaf damage.
12. Initial insect bite and nonfeeding of insect was observed in the clone GRB 1-06, 2-18 and 5-15 and the magnitude of leaf damage was estimated to be 10%, 5% , 20% respectively and 50% for the plant 'Himalaya'.
13. So the clone GRB 2-18 was finally selected for field trials against the check varieties (Gomti, Kalka, Kosi, Himalaya and MAS-1) which showed vigorous growth and better yield of herbage and oil .

Comparison of Sambhav with the check varieties

The new genotype GRB 2-18 named as 'Sambhav' was so rapidly growing that it could cover the inter-row spacing of 80cm completely within a period of 90-100 days, which was not the case of other control varieties taken (Drawing sheet # 2, Photograph # 1 and 2). The plant canopy covered a space of 85 to 90 cm in 110 days in comparison to Himalaya(62-70 cm), Shivalik (50-56 cm), Gomti (70-75), Kosi (65-72), Kalka (40-60 cm) and MAS-1(40-50 cm). The herbage yield of the plant 2.8 Q per 100 m^2 against 1.25, 0.75, 0.50, 0.80, 1.60, 1.70 for Himalaya, Shivalik, MAS-1, Kalka, Gomti and Kosi. Similarly the oil yield was highest (0.8%) in case of the genotype. The genotype though has less menthol percentage, when the total menthol yield per 100 m^2 is estimated by converting the total oil yield per 100 m^2 against the menthol percentage (77 X 1.61 / 100) and it comes to 1.24 kg, which is much higher

than other varieties. The menthol yield values for other varieties are 0.68, 0.27, 0.24, 0.52, 0.60,1.08 kg per 100 m^2 for Himalaya, Shivalik, MAS-1, Kalka, Gomti and Kosi respectively. This was the most desirable economical advantage to the plant with commercial value as it produced highest amount of herbage and ultimately the oil and menthol per unit area in comparison to other genotypes while being the most tolerant to insect pest attack.

CLAIMS

1. A novel screening method useful for the selection and development of an insect tolerant genotypes or clones, said method comprising the steps of:

   a) generating the clones of an insect tolerant plant through tissue culture as somaclones and establishing their molecular distinctiveness prior to screening through RAPD analysis at in *vitro* stage itself;

   b) micro-propagating the identified molecular variants for multiplication and checking for the stability at molecular level through RAPD among clones of larger population,

   c) transferring the identified stable variants after being multiplied to individual culture tubes for forced feeding of insects by releasing actively feeding larvae or nymphs into each culture tube, and

   d) multiplying in-vitro the surviving clones and rechecking for insect larval non-preference and then field evaluating under natural or artificial insect infestation conditions.

2. A novel screening method as claimed in claim 1 wherein the plants are raised by somacloning or rapid propagation method.

3. A novel screening method useful for the selection and development of an insect tolerant mint plant genotypes or clones, said method comprising the steps of:

   a) generating the clones of an insect tolerant plant through tissue culture as somaclones and establishing their molecular distinctiveness prior to screening through RAPD analysis at in *vitro* stage itself;

b) micro-propagating the identified molecular variants for multiplication and checking for the stability at molecular level through RAPD among clones of larger population,

c) transferring the identified stable variants after being multiplied to individual culture tubes for forced feeding of insects by releasing actively feeding larvae or nymphs into each culture tube, and

d) multiplying in-vitro the surviving clones and rechecking for insect larval non-preference and then field evaluating under natural or artificial insect infestation conditions.

4. A screening method as claimed in claim 1 or 3 wherein the insect tolerance trait of the plant is not limited to *S-obliqua* and may cover other insect pests which feed on foliage of the plant.

5. A screening method as claimed in claim 1 or 3 wherein the clones could be generated vegetatively, tissue culture, glass house or in field by asexual reproduction method.

Abstract

A novel screening method useful for the selection and development of an insect tolerant genotypes or clones, said method comprising the steps of : (a) generating the clones of an insect tolerant plant through tissue culture as somaclones and establishing their molecular distinctiveness prior to screening through RAPD analysis at in *vitro* stage itself; b) micro-propagating the identified molecular variants for multiplication and checking for the stability at molecular level through RAPD among clones of larger population, c) transferring the identified stable variants after being multiplied to individual culture tubes for forced feeding of insects by releasing actively feeding larvae or nymphs into each culture tube, and d) multiplying in-vitro the surviving clones and rechecking for insect larval non-preference and then field evaluating under natural or artificial insect infestation conditions.

Practitioner's Docket No. U 012567-2 *PATENT*

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: SUMAN PREET **SINGH KHANUJA**, et al
Application No.: Group No.:
Filed: Examiner:
For: A NOVEL SCREENING METHOD FOR SELECTION OF INSECT TOLERANT PLANTS

Assistant Commissioner for Patents
Washington, D.C. 20231
ATTENTION: Deputy Assistant Commissioner for Patents

PETITION TO ACCEPT COLOR DRAWINGS OR PHOTOGRAPHS (37 CFR 1.84(a)(2) and (b)(2))

1. This petition is for the acceptance of color:

   [ ] drawings (37 C.F.R. § 1.84(a)(2))

   [X] photographs (37 C.F.R. § 1.84(b)(2))

2. Attached hereto are three (3) sets of color: [ ] drawings [X] photographs.

3. Please amend the specification, by inserting the following language as the first paragraph of the specification beginning a brief description of the drawing or photograph (page 3 between lines 1 and 2).

   "The file of this patent contains at least one drawing or photograph executed in color. Copies of this patent with color drawing(s) or photograph(s) will be provided by the Patent and Trademark Office upon request and payment of necessary fee."

4. The reason(s) for the need for color drawings or photographs in this application is/are as follows:

5. The petition fee required to waive the requirements of § 1.84 (37 C.F.R. § 1.17(i)) is paid as follows:

[X] Attached is a check for the sum of $130.00.
[ ] Charge account _____ the sum of $130.00.
A duplicate of this petition is attached.

SIGNATURE OF PRACTITIONER

Reg. No. 33,778

JANET I. CORD
(type or print name of practitioner)

Tel. No.: (212)708-1935

LADAS & PARRY
P.O. Address

Customer No.:

26 WEST 61ST STREET
NEW YORK, NEW YORK 10023

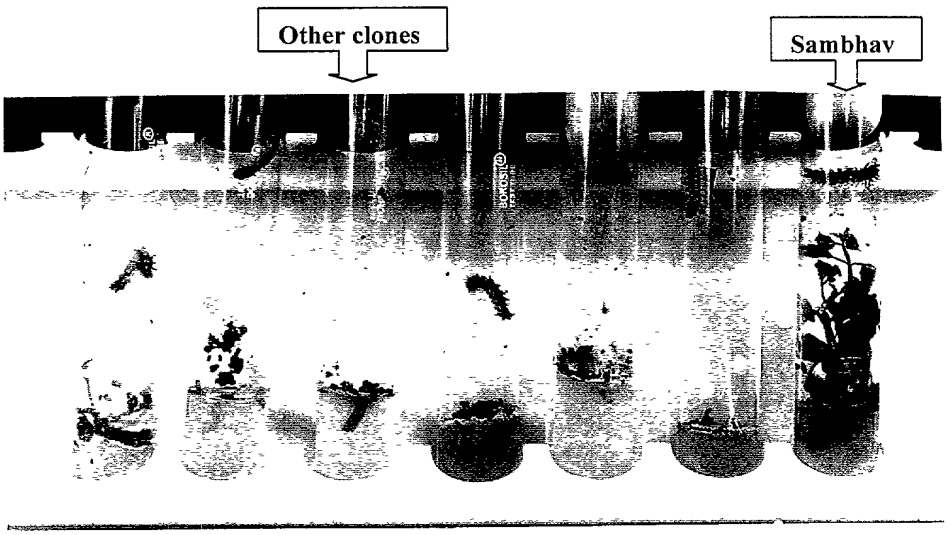
Photograph # 1: Field view of 70 days old crop

Photograph # 2: Field view of 100 days old crop

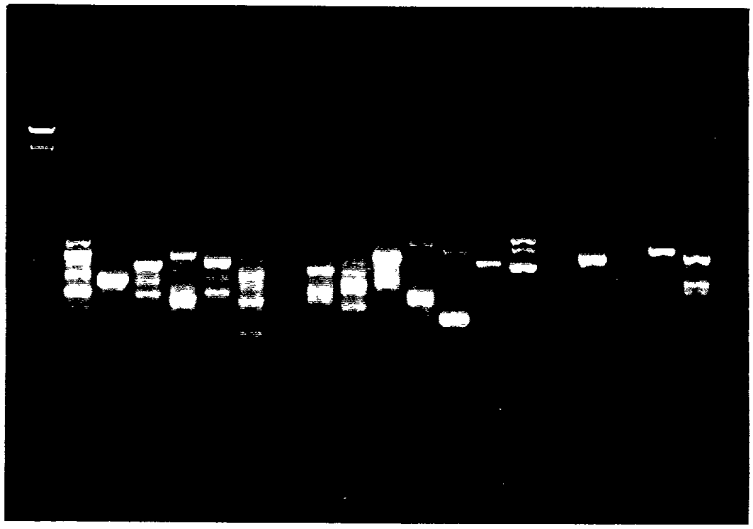
Photograph # 1 : A 70 days old plant

Photograph # 2: A 100 days old plant

SHEET No. 3



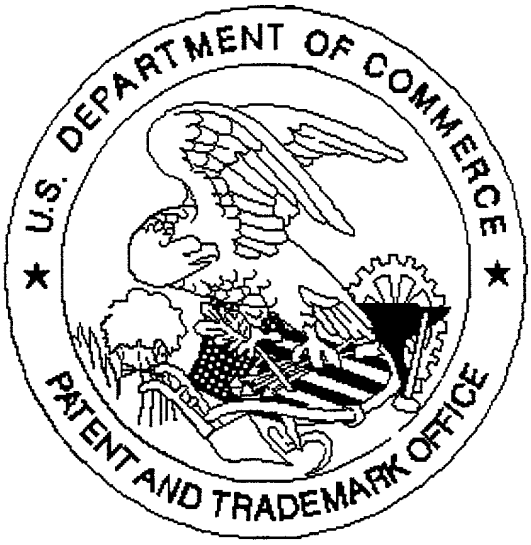
Photograph # 1 : Forced larval feeding test for selection of clones



Photograph # 2 : Unique RAPD Profile of "Sambhav"

United States Patent & Trademark Office

Office of Initial Patent Examination -- Scanning Division



Application deficiencies were found during scanning:

☑ Page(s)_____ of 6 Drawing Sheets (missing) were not present for scanning. (Document title)

☑ Page(s) 10 of Spec. were not present for scanning. (Document title)

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